Supporting Information for

JNK-mediated phosphorylation of SARM1 regulates NAD^+ cleavage activity to inhibit mitochondrial respiration

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This PDF file includes:

Figs. S1 to S5

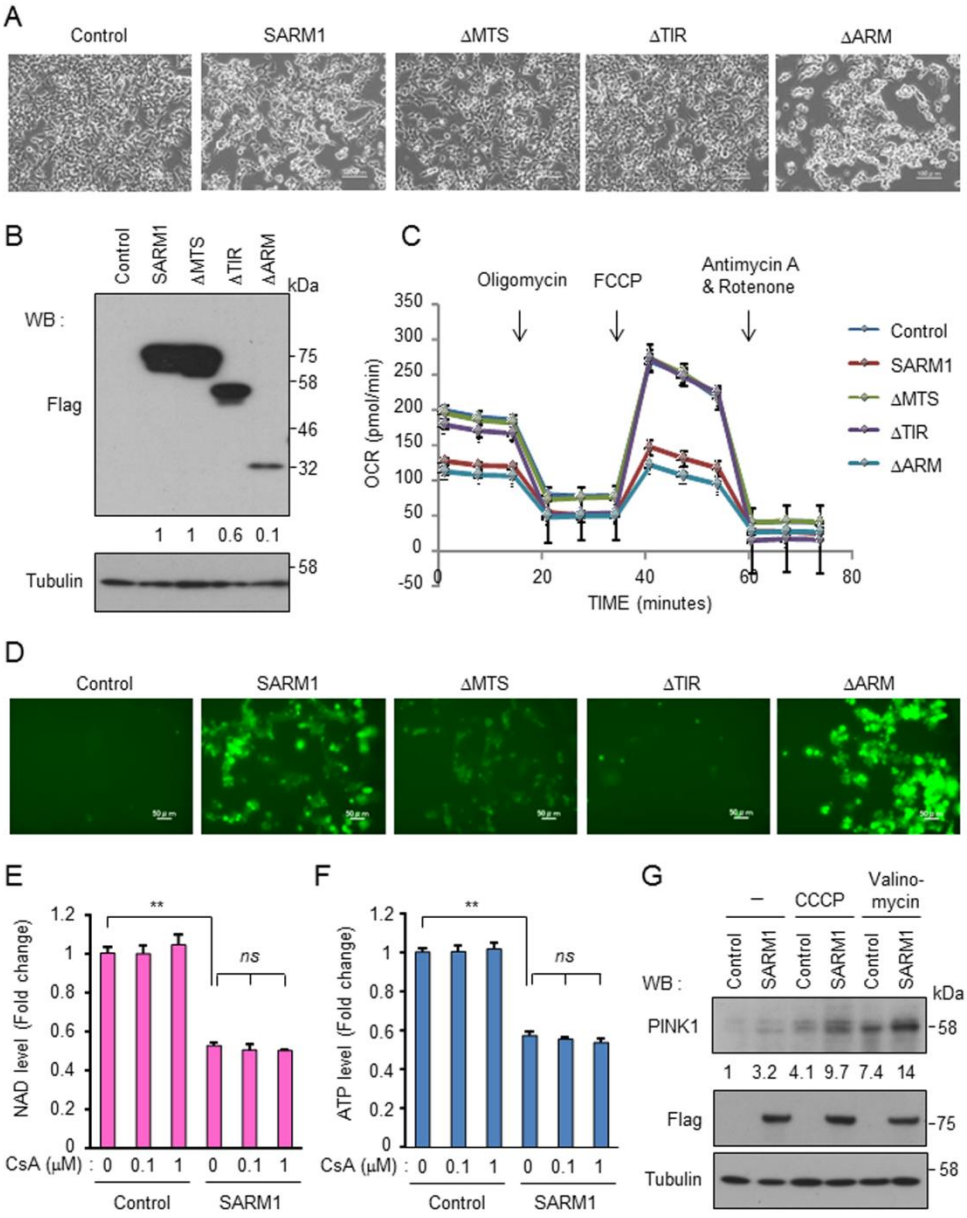

Figure S1. SARM1 overexpression hampers mitochondrial function. (A) Phase contrast micrographs of HEK293T cells expressing SARM1 constructs. Bar, 100 μm. (B) Expression level of SARM1 constructs in HEK293T cells. Flag-tagged SARM1 constructs were densitometrically quantified (Flag/ Tubulin). (C) To analyze OCR, 1.5 μM oligomycin, 0.25 μM FCCP and 0.5 μM rotenone/antimycin A were used. (D) ROS production was analyzed by CM-H_2DCFDA staining. Bar, 50 μm. (E and F) Cyclosporin A (CsA), a PTP inhibitor, did not affect SARM1-induced NAD^+ and ATP reduction. (G) SARM1 overexpression enhances PINK1 accumulation on depolarized mitochondria. HEK293T cells were transfected with pDNA. At 24 h post-transfection, the cells were treated with 10 μM CCCP or 10 μM valinomycin and further incubated for 3 h. PINK1 were quantified by densitometry (PINK1/ Tubulin).

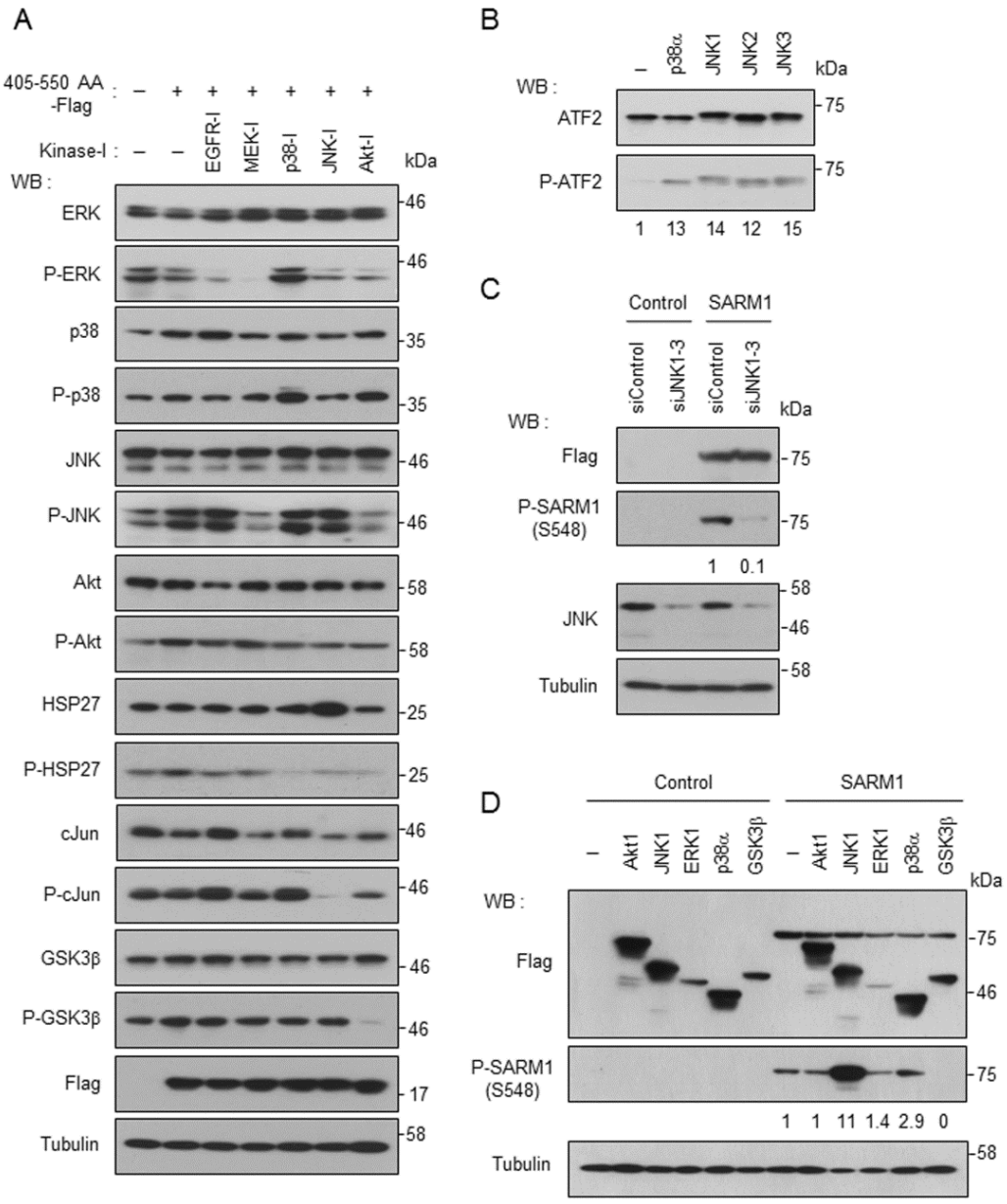

Figure S2. JNK regulates SARM1 phosphorylation. (A) Confirmation of the inhibitory effect of kinase inhibitors. The experiment was performed under same conditions to those in Fig. 3A. (B) Confirmation of the catalytic activity of kinases. (C) Down-regulation of SARM1 phosphorylation by knock-down of JNK. Control siRNA or JNK siRNAs (mixtures of siRNA targeting JNK1, 2 and 3) was transfected in HEK293T cells. At 24 h post-transfection, the cells were transfected with control or SARM1-Flag pDNA and further incubated for 24 h. (D) Up-regulation of SARM1 phosphorylation by overexpression of JNK. The protein expression was confirmed by anti-Flag antibody. Phosphorylated proteins were quantified by densitometry (Phosphorylated protein/ total protein).

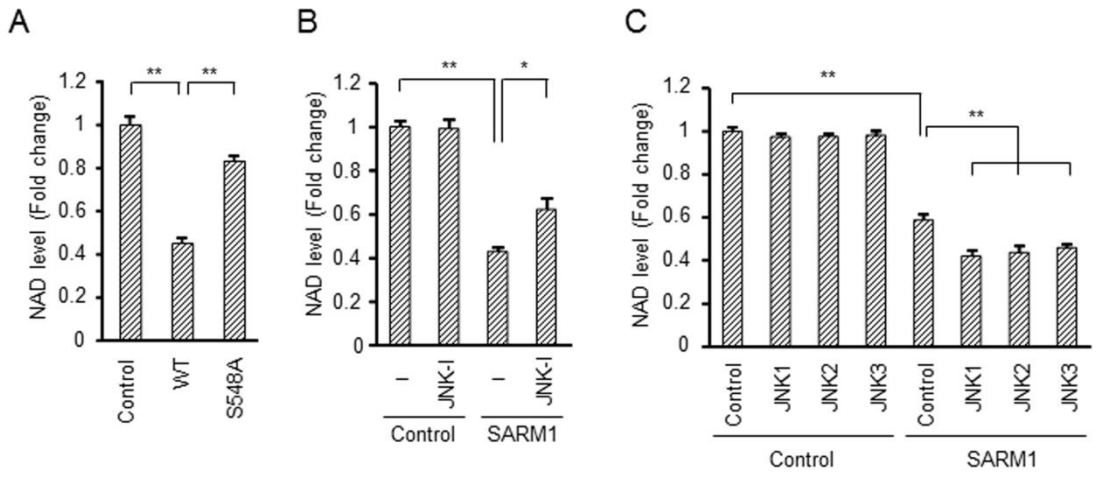

Figure S3. JNK-mediated phosphorylation of SARM1 affects intracellular NAD^+ level. (A-C) The indicated constructs were transfected in HEK293T cells for 24 h. the intracellular NAD^+ levels were measured by the NAD/NADH-Glo assay.

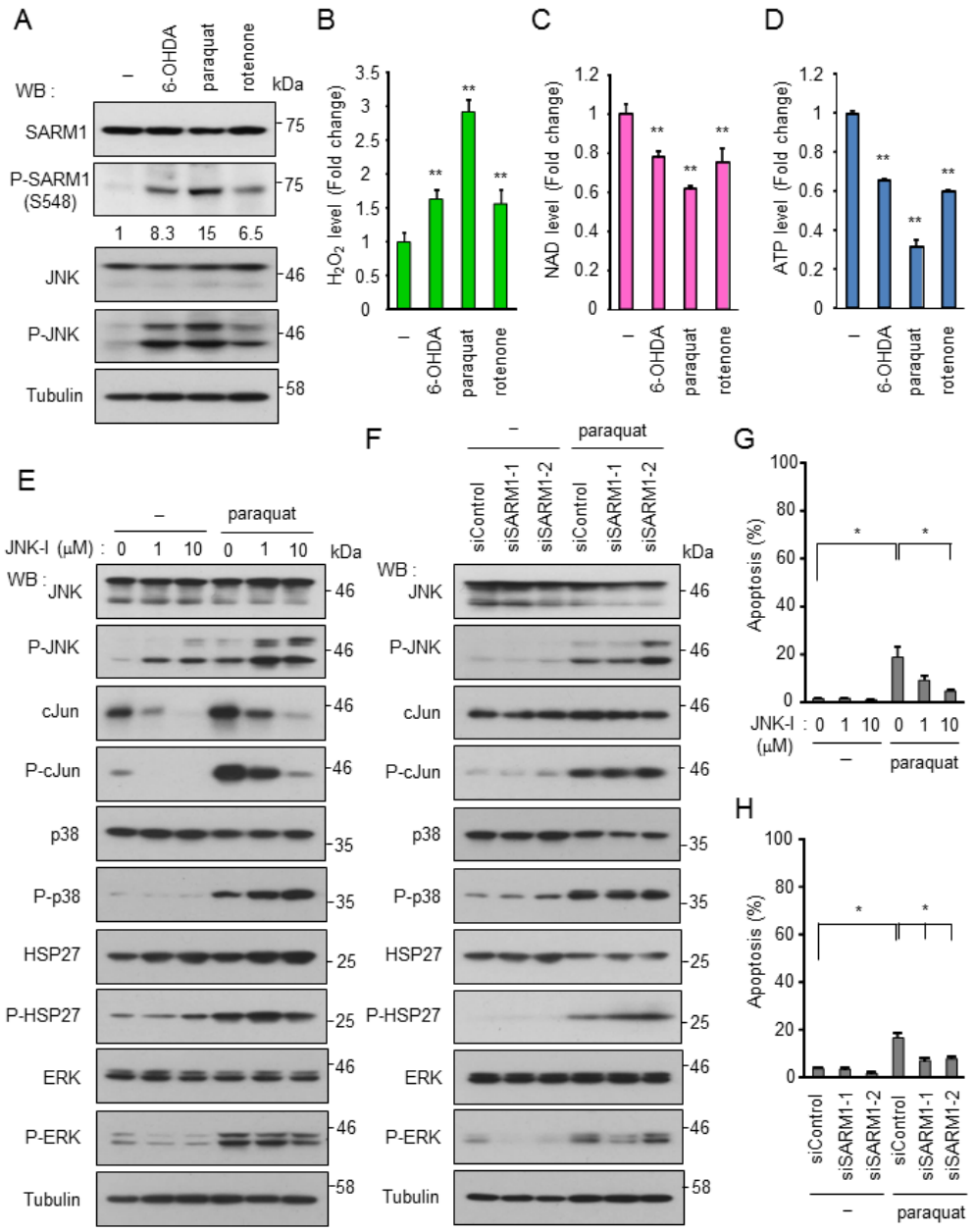

Figure S4. Induction of SARM1 phosphorylation by oxidative stresses. (A) Detection of endogenous SARM1 phosphorylation. SH-SY5Y cells were treated with 20 μM 6-OHDA, 1 mM paraquat or 1 μM rotenone for 24 h. (B-D) Oxidative stresses change levels of ROS, NAD^+ and ATP. (E) Phosphorylation of MAPK cascade proteins under paraquat and JNK-I treatment conditions. The experiment was performed under same conditions to those in Fig. 5A. (F) Phosphorylation of MAPK cascade proteins under paraquat treatment and SARM1 down-regulation conditions. The experiment was performed under same conditions to those in Fig. 5D. (G and H) Apoptotic cells were identified after staining with Hoechst33342. *, $p < 0.05$; **, $p < 0.01$.

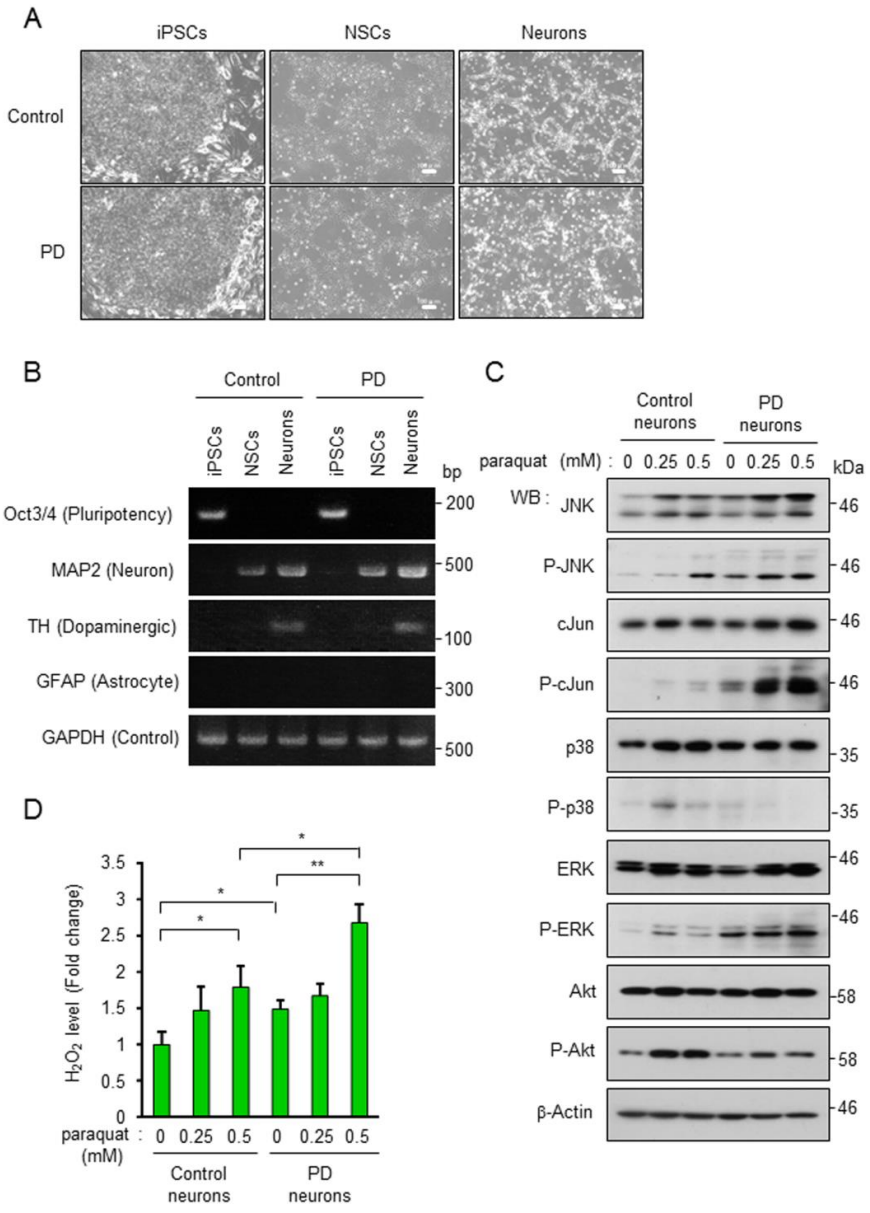

Figure S5. Induction of NSCs and matured neurons from human control iPS cells and PD patient-derived iPS cells. (A) Representative images of control and PD-derived iPSCs, NSCs and neurons. Bar, 100 μm. (B) Marker analysis of iPSCs, NSCs and neurons. RT-PCR analysis showed almost the same expression levels of pluripotency markers Oct3/4 in iPSC, neuronal marker MAP2 in NSC and neurons, and dopaminergic neuron marker TH in neurons between control and PD-derived cells. The astrocyte marker GFAP showed no expression and GADPH was used as a control. (C) Phosphorylation of MAPK cascade proteins of neurons under paraquat treatment conditions. (D) H_2O_2 levels of Control neurons and PD neurons under paraquat treatment conditions. *, $p < 0.05$; **, $p < 0.01$.